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Is *Liriomyza langei* a real species or a biotype of *L. huidobrensis*?



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Is *Liriomyza langei* a real species or a biotype¹ of *L. huidobrensis*?

Liriomyza huidobrensis is a leafminer that originated in Latin America and has since established populations in temperate and subtropical regions worldwide. There is evidence that a population found in California is not merely a biotype of *L. huidobrensis* but a distinct species called *L. langei*. While the Latin American and Californian populations cannot be differentiated based on morphology, differences in their invasive histories, host preferences, and feeding and oviposition behavior suggest they may have evolved into different species. Furthermore, molecular evidence and a possible reproductive isolating mechanism indicate that the two populations are individual species. This document discusses evidence that the Californian population may be distinct species rather than a biotype of *L. huidobrensis*.

What is a species?

From the time of Plato and Aristotle until Linnaeus, the word “species” simply meant a class of a class of objects that shared certain defining properties (Mayr, 1996). Philosophers applied this definition, which is often referred to as the morphological or typological species concept, to a wide range of animate and inanimate objects (Mayr, 1996). Naturalists, during this period, recognized biological species by morphological differences (Mayr, 1996).

Some early prophetic naturalists, like Buffon and Darwin, hinted at the biological species concept, but the concept was not clearly articulated until the late 19th and early 20th century, when studies revealed that similar looking “species” could behave very differently (Mayr, 1996). Widespread recognition of the many cryptic species that occur in nature, and recognition that many morphotypes can occur in a single species because individuals differ in sex, age, or genetics, eventually led to the almost complete replacement of the typological species concept by the biological species concept (BSC) (Mayr, 1996).

According to Ernst Mayr (1996), a staunch supporter of the BSC and critic of those who try to redefine it, “species are groups of interbreeding natural populations that are reproductively isolated from other such groups.” The isolating mechanism is a property of individuals, so geographic isolation, by itself, does not qualify populations as distinct species. Biologists must ask themselves whether or not their study population would interbreed with geographically isolated populations should the populations meet in nature. The biologist must then use morphology, geography, ecology, behavior, and molecular information, along with inference because “during a period of geographic isolation the presence of species specific isolating mechanisms can only be inferred,” to answer the question and demarcate species.

Do we have enough information to demarcate populations of *Liriomyza huidobrensis* that originate in either California or Latin America as separate species?

Unequal rates of evolution and a lack of information on the mating potential of isolated populations have always been problems for the BSC and species demarcation (Mayr, 1996). Biologists should expect to find populations in nature that are on their way to becoming new species because evolution is a gradual process (Mayr, 1996). Spencer predicted speciation of polyphagous agromyzid populations in 1973. He speculated that polyphagy arose independently only twice in the genus *Liriomyza*, and that the five known polyphagous species would, with some degree of isolation and host plant restriction, diverge into new “more typical monophagous or oligophagous” species; the vast majority of agromyzids are host specialists with less than one percent regularly feeding on more than two plants in different taxonomic orders or subclasses (Spencer, 1990). Speciation may actually be occurring in Californian populations of *L. huidobrensis*. Morgan *et al.* (2000) did not find any evidence of genetic mixing between central and southern Californian populations, and Reitz and Trumble (2002) concluded that the populations were distinct biotypes based on differential reproductive success rates on a variety of hosts and a higher frequency of homotypic than heterotypic mating in laboratory studies. The following sections of this paper discuss what science knows about Californian and Latin American *L. huidobrensis* populations in terms of what a biologist can use to demarcate species.

Morphology

Adult members of the genus *Liriomyza* all look very similar; they are small black flies that often have bright yellow patterns on their scutellum (EPPO, 2005). Species identification is difficult and often relies on a close examination

¹ Biologists can not designate either the Californian or Latin American population as an incipient species or a subspecies of *L. huidobrensis* because there are no known morphological differences in the two populations.

Attachment 2-SPRO-CPHST report

of small external differences, such as the relative lengths of wing veins, the presence and position of a particular seta, or the color of the cuticle from which a seta arises (EPPO, 2005; Masetti *et al.*, 2006; Weintraub and Horowitz, 1995). Identification of closely related *Liriomyza* species is only possible by examining male genitalia (EPPO, 2005; Masetti *et al.*, 2006; Shiao, 2004). Even then, it is difficult to distinguish species; the genitalia must be properly prepared and viewed from the correct angle by an experienced identifier (Shiao, 2004; Shiao and Wu, 2000).

The immature stages of agromyzid flies are even more difficult to identify than the adults. The eggs can not even be identified to genus (EPPO, 2005). The larvae and pupae can be separated into species groups by morphological characteristics, but species determination requires electrophoretic or molecular analysis (EPPO, 2005).

There are no known morphological differences between members of the Californian and Latin American *L. huidobrensis* populations (Scheffer, 2000; Scheffer and Lewis, 2001; Scheffer *et al.*, 2001). Shiao (2004) recently evaluated the usefulness of some morphological characters to separate *Liriomyza* species of quarantine importance to Taiwan. He included specimens from California in his study of abdominal color patterns but found no differences between the Californian and Taiwanese *L. huidobrensis* populations. He did not include the Californian specimens in his study of thoracic microsetae, and it is not clear if he included them in the wing morphometrics or genitalia studies.

Geography

Molecular evidence suggests that the Californian and Latin American populations diverged about two million years ago (Scheffer, 2000). Since then, the Latin American population has spread to temperate and subtropical regions throughout the world (Chen and Kang, 2004; He *et al.*, 2002; Scheffer, 2000; Scheffer and Lewis, 2001; Scheffer *et al.*, 2001). In sharp contrast, the Californian population, which is frequently intercepted in Florida on vegetables from California (Halbert, 2006), has not spread since damage was reported by Lange *et al.* in 1957 (Borchert, 2006). The fly was introduced into Hawaii from California and established before 1952 (Hardy and Delfinado, 1980; Scheffer, 2000; Spencer, 1973). It was first detected in Washington state in 1918, where it is still occasionally classified as a serious pest (Gary *et al.*, 1986); the last reported outbreak occurred in 2003 (Pelter, 2003). Specimens in the Oregon State Arthropod Collection suggest that this fly was introduced into Oregon before 1938 (Marshall, 2007). Stegmaier (1968) listed the U.S. distribution as California, Oregon, and Washington. Gary *et al.* (1986) listed the U.S. distribution as California, Florida, Utah, and Virginia. Poe and Montz (1981) recorded the fly's presence in California, Virginia, and Florida. Steck and Dixon (2006) explained that the fly never established a permanent population in Florida, and CABI (2006) states that it is now absent from Florida, Utah, and Virginia.

Differences in the invasive histories of the two populations could be explained a number of ways but almost certainly involve differences in their abilities to survive diverse climatic conditions; introduction into areas with unfavorable climates is one of the leading reasons that introduced organisms fail to establish (Lodge, 1993), and scientists believe that thermal adaptation is a heritable characteristic (Chen and Kang, 2004).

Chen and Kang (2004) proved that Chinese populations, which originated from Latin American stock (He *et al.*, 2002), could survive cold temperatures and extend their range through supercooling. The absolute minimum temperatures that tested pupae survived increased with latitude from $\approx -18^{\circ}\text{C}$ at 25° latitude to $\approx -21^{\circ}\text{C}$ at 31 to 34° latitude (Chen and Kang, 2004). Because an insect's supercooling point is not a reliable indicator of cold hardiness (Martin *et al.*, 2005a), Chen and Kang (2004) estimated that members of the Chinese populations could not over-winter in areas where the mean monthly temperature was near -5°C , based on lethal time and temperature studies. Martin *et al.* (2005a) supported Chen and Kang's (2004) estimate by demonstrating that members of a Canadian population, which also originated from Latin American stock (Scheffer *et al.*, 2001), could not survive 16 consecutive days at -5°C . Some authors, who studied *L. huidobrensis* cold hardiness, recognized color variation in the puparia and suggested that dark colored puparia may over-winter in colder areas than light colored puparia (Weintraub and Horowitz, 1995). Other authors, who studied descendants of Latin American populations, suggest that all life stages are fairly cold tolerant (Lanzoni *et al.*, 2002), but Lange *et al.* (1957) indicate that cold winters tend to decrease fly populations in the Salinas Valley of California, where the mean low temperature during the coldest winter month is about 2°C (NOAA, 2007).

Although researchers report low *L. huidobrensis* populations, less female activity, and low fecundity at temperatures above 30°C (Weintraub, 2001), members of the Latin American population may adapt to warm climates. Lanzoni *et al.* (2002) showed that an Italian population, which originated from Latin American stock, could not complete development at a constant temperature of 30°C , but the authors recognized that other populations, such as those in

Attachment 2-SPRO-CPHST report

Reunion, which probably also originated from Latin American stock, may complete development at this temperature. The diverse climatic situations that Latin American populations experienced as they migrated south along the Andes to Argentina (Spencer, 1973) may have equipped individuals with thermal adaptations that are not found in members of the Californian population.

Ecology

Ecology is a broad field that studies the interactions between and among organisms and their environment. As such, many ecological interactions are discussed throughout this paper. One ecological topic of paramount importance that has not yet been discussed is plant-herbivore interactions. For this reason, the following section concentrates on known differences in the host ranges of Californian and Latin American *L. huidobrensis* populations.

Interactions between *Liriomyza* species and their hosts are complex (Wei *et al.*, 2000). Their host ranges appear to be determined by female feeding and oviposition preferences rather than the host's suitability for larval survival and development (Reitz and Trumble, 2002). For example, Martin *et al.* (2005b) suggested that members of a Canadian *L. huidobrensis* population, which originated from Latin American stock (Scheffer *et al.*, 2001), prefer cucumber (*Cucumis sativus* cv. Calypso) to lettuce (*Lactuca sativa* cv. Ithaca) based on the proportion of eggs in feeding and oviposition punctures from three subpopulations, but the authors also demonstrated that larvae develop significantly faster and grow into larger pupae on lettuce than on cucumber. Wei *et al.* (2000) explained *L. huidobrensis* host selection in terms of leaf morphology; they suggested that females prefer to feed from and oviposit in leaves that have a thin epidermis and a low density mesophyll layer. These conditions make it easy for the female to puncture a leaf with her ovipositor (Wei *et al.*, 2000).

The primary literature contains numerous records of members or recent descendants of the Latin American population attacking crops, ornamentals, and weeds under natural conditions that are not known hosts of the Californian population. Some of these plants, such as Indian aster (*Kalimeris indica*), sponge gourd (*Luffa aegyptiaca*) (Shiao and Wu, 2000), and Ceylon spinach (*Basella albe*) (Rauf *et al.*, 2000) do not occur in areas of California where *L. huidobrensis* is present (Borchert, 2006; CDFA, 2006; NASS, 2007; USDA-NRCS, 2007). Other recorded natural hosts of the Latin American population are present in California and apparently unattractive to the Californian population; research and extension personnel in California did not indicate that any of the plants listed in Table 1 are attacked by *L. huidobrensis* (Colpetzer, 2007).

Table 1: Plants attacked under natural conditions by members or descendants of the Latin American population that are not known hosts of the Californian population

Plant	Country	Reference
<u>Asteraceae</u>		
<i>Ageratum conyzoides</i> (tropical whiteweed)	Indonesia	Rauf <i>et al.</i> , 2000
<i>Ageratum</i> sp. (whiteweed)	Indonesia	Shepard <i>et al.</i> , 1998
<i>Arctium lappa</i> (greater burdock)	Philippines	Scheffer <i>et al.</i> , 2006
<i>Calendula</i> sp. (marigold)	Chile	Spencer, 1990
<i>Conyza canadensis</i> (Canadian horsetail)	China	Wei <i>et al.</i> , 2000
<i>Dahlia</i> sp. (dahlia)	Indonesia Venezuela	Rauf <i>et al.</i> , 2000 Spencer, 1973
<i>Emilia sonchifolia</i> (lilac tasselflower)	Indonesia Philippines Sri Lanka	Rauf <i>et al.</i> , 2000 Scheffer <i>et al.</i> , 2006 Scheffer <i>et al.</i> , 2001
<i>Erechtites hieraciifolia</i> (American burnweed)	Indonesia	Rauf <i>et al.</i> , 2000
<i>Galinsonga parviflora</i> (gallant soldier)	China	Wei <i>et al.</i> , 2000
<i>Galinsonga quadriradiata</i> (shaggy soldier)	Venezuela	Spencer, 1973
<i>Ganzania</i> sp.(ganzania)	Colombia Europe South America	Spencer, 1990 Spencer, 1990 Spencer, 1990
<i>Gerbera jamesonii</i> (Barberton daisy)	Italy	Lanzoni <i>et al.</i> , 2002

Attachment 2-SPRO-CPHST report

Plant	Country	Reference
	Indonesia	Rauf <i>et al.</i> , 2000
	China	Wei <i>et al.</i> , 2000
	Lebanon	Hammad <i>et al.</i> , 2000
	Poland	Górski, 2005
<i>Luecanthemum</i> sp. (daisy)	Netherlands	Kox <i>et al.</i> , 2005
<i>Tagetes erecta</i> (Aztec marigold)	China	Wei <i>et al.</i> , 2000
<i>Tagetes patula</i> (French marigold)	China	Wei <i>et al.</i> , 2000
<i>Tagetes</i> sp. (marigold)	Argentina	Spencer, 1990
<u>Brassicaceae</u>		
<i>Barbarea</i> sp. (yellowrocket)	Taiwan	Shiao and Wu, 2000
<i>Nasturtium officinale</i> (watercress)	Indonesia	Rauf <i>et al.</i> , 2000
<u>Caryophyllaceae</u>		
<i>Stellaria media</i> (common chickweed)	China	Wei <i>et al.</i> , 2000
<u>Convolvulaceae</u>		
<i>Calystegia sepium</i> (hedge false bindweed)	China	Wei <i>et al.</i> , 2000
<i>Ipomoea batatas</i> (sweetpotato)	Indonesia Indonesia	Shepard <i>et al.</i> , 1998 Rauf <i>et al.</i> , 2000
<u>Cucurbitaceae</u>		
<i>Sechium edule</i> (chayote)	Indonesia	Rauf <i>et al.</i> , 2000
<u>Fabaceae</u>		
<i>Lathyrus odoratus</i> (sweetpea)	Argentina	Spencer, 1973
<i>Vigna unguiculata</i> (blackeyed pea)	China Indonesia Indonesia	Wei <i>et al.</i> , 2000 Shepard <i>et al.</i> , 1998 Rauf <i>et al.</i> , 2000
<u>Gentianaceae</u>		
<i>Eustoma exaltatum</i> ssp. <i>russellianum</i> (showy prairie gentian)	China	Wei <i>et al.</i> , 2000
<u>Iridaceae</u>		
<i>Gladiolus hybridus</i> (gladiolus)	China	Wei <i>et al.</i> , 2000
<u>Lamiaceae</u>		
<i>Ocimum basilicum</i> (sweet basil)	Indonesia	Rauf <i>et al.</i> , 2000
<u>Malvaceae</u>		
<i>Malva verticillata</i> (cluster mallow)	China	Wei <i>et al.</i> , 2000
<i>Sida</i> sp. (fanpetals)	Philippines	Scheffer <i>et al.</i> , 2006
<u>Oxalidaceae</u>		
<i>Oxalis</i> sp. (woodsorrel)	Argentina	Spencer, 1990
<u>Poaceae</u>		
<i>Setaria viridis</i> (green bristlegrass)	China	Wei <i>et al.</i> , 2000
<u>Polemoniaceae</u>		
<i>Phlox</i> sp. (phlox)	Argentina	Spencer, 1973
<u>Ranunculaceae</u>		
<i>Ranunculus sceleratus</i> (cursed buttercup)	China	Wei <i>et al.</i> , 2000

Attachment 2-SPRO-CPHST report

Plant	Country	Reference
<u>Scrophulariaceae</u>		
<i>Antirrhinum majus</i> (garden snapdragon)	Canada	Martin <i>et al.</i> , 2005b
<u>Solanaceae</u>		
<i>Datura</i> sp. (jimsonweed)	South America	Spencer, 1990
<i>Physalis angulata</i> (cutleaf groundcherry)	Indonesia	Rauf <i>et al.</i> , 2000
<i>Solanum americanum</i> (American black nightshade)	Indonesia	Rauf <i>et al.</i> , 2000
<i>Solanum tuberosum</i> (Irish potato)	Canada	Martin <i>et al.</i> , 2005b
	Indonesia	Shepard <i>et al.</i> , 1998
<i>Solanum tuberosum</i> (Irish potato) continued	Indonesia	Rauf <i>et al.</i> , 2000
	Indonesia	Hidayani <i>et al.</i> , 2005
	Israel	Weintraub and Horowitz, 1996
	Israel	Weintraub, 2001
	South Africa	Scheffer <i>et al.</i> , 2001
	Venezuela	Spencer, 1973
<u>Tropaeolaceae</u>		
<i>Tropaeolum</i> sp. (nausturtium)	Argentina	Spencer, 1973
<u>Violaceae</u>		
<i>Viola</i> sp. (violet)	Argentina	Spencer, 1973

Some researchers recognized that the Californian and Latin American populations seemed to differ in host plant preference and insecticide resistance (Lanzoni *et al.*, 2002; Scheffer, 2000; Scheffer and Lewis, 2001; Scheffer *et al.*, 2001); the Californian population is “relatively easily controlled,” while pesticide resistant strains occur in other areas of the world (Lanzoni *et al.*, 2002). The over use of pesticides in South America during the 1970s is supposedly responsible for the resistant strains (Lanzoni *et al.*, 2002), but it may also partially explain why the Latin American population is recorded on a wider range of hosts than the Californian population. The Latin American population may have evolved either more efficient or different detoxification mechanisms than the Californian population due to extreme evolutionary pressures. These new or improved detoxification mechanisms could allow individuals to exploit a wide range of hosts with very diverse phytochemicals.

Behavior

All *Liriomyza* adults exhibit similar feeding behavior (Ameixa *et al.*, 2007; Parrella, 1987), but their larvae make characteristic leaf mines, and individual species differ in how they exploit the mesophyll layer (Parrella *et al.*, 1985). The mining behavior of an individual species may vary on different hosts, but numerous observations of *L. huidobrensis* in California suggest that the Californian population prefers to mine the spongy mesophyll layer of chrysanthemum leaves (Parrella and Bethke, 1984; Parrella *et al.*, 1985). In stark contrast to the Californian population, Spencer (1973) observed that *L. huidobrensis* mines occur with “almost equal frequency” in the upper (*i.e.*, palisade mesophyll) and lower (*i.e.*, spongy mesophyll) layers of chrysanthemum leaves in Timotes, Venezuela. Numerous conditions, including high population pressures, could have influenced the mining behavior that Spencer observed in Venezuela, or the Latin American population may actually have a slightly different mining behavior than the Californian population. EPPO (2005) supports Spencer’s observation by stating that *L. huidobrensis* mines undulate between upper and lower leaf surfaces. Additional scientific literature from around the world does not help answer whether different populations exhibit different mining behaviors because most publications either directly (*e.g.*, Weintraub and Horowitz (1996)) or indirectly (*e.g.*, Civelek *et al.* (2004)) regurgitate the findings of Parrella *et al.* (1985).

Female oviposition behavior is another potential difference between the Californian and Latin American populations. Blanchard (1926) wrote in the original description of *L. huidobrensis* from *Cineraria* in Argentina that, “females deposit small white oval eggs in the tissues of the leaf from the underside.” Weintraub and Horowitz (1995) also state that female *L. huidobrensis* deposit eggs into the lower surface of leaves, but it is not clear whether they are citing another researcher’s work, or stating what they observed in an Israeli population that originated from Latin American stock (Scheffer, 2000; Scheffer and Lewis, 2001). In sharp contrast, Parrella and Bethke (1984)

Attachment 2-SPRO-CPHST report

showed that female *L. huidobrensis* from California make more feeding and oviposition punctures on the upper than lower surfaces of aster, chrysanthemum, and pea leaves. This fact is reflected in their observation that a larval mine “usually begins on the upper surface and moves to the lower surface after a few millimeters” (Parrella and Bethke, 1984). This behavioral difference may be real, or it may be an artifact of observations on different host plants.

Molecular Information

In 2000, Scheffer published a paper that analyzed a 941 base pair segment of the mitochondrial cytochrome oxidase I and II genes from various *L. huidobrensis* populations. Scheffer included specimens from various host plants and various locations (*i.e.*, California, Ecuador, Guatemala, Hawaii, Indonesia, Israel, and Sri Lanka) in this study, and from the results, she suggested that *L. huidobrensis*, as currently defined, contains, at least, two cryptic species. She suggested this because maximum parsimony analysis sorted the DNA sequence data into two well-defined monophyletic clades that differed in sequence divergence by a magnitude found in other agromyzid species; one clade contained sequence data from Californian and Hawaiian specimens, while the other contained sequence data from Latin American and non-American specimens. As mentioned above, Scheffer used a generalized insect mitochondrial DNA molecular clock estimate of 2.3% sequence divergence per million years to estimate that the Californian and Latin American populations diverged about two million years ago.

In 2001, Scheffer and Lewis published a paper that analyzed segments of two nuclear genes (*i.e.*, a 171 base pair segment of β -tubulin and a 921 base pair segment of elongation factor-1 α) from various *L. huidobrensis* populations. They also included specimens from various host plants and various locations (*i.e.*, Argentina, California, Colombia, Ecuador, Guatemala, Hawaii, Indonesia, Israel, Peru, and Sri Lanka) in their study. Again, maximum parsimony analysis sorted the DNA sequence data into two well-defined monophyletic clades (*i.e.*, one clade contained sequence data from Californian and Hawaiian specimens, while the other contained sequence data from Latin American and non-American specimens). Scheffer and Lewis (2001) used these results to formally resurrect the name *Liriomyza langei* for flies belonging to the Californian/Hawaiian clade and restricted the name *L. huidobrensis* to flies belonging to the Latin American clade. They did this because analyses of the sequence data from three independent gene regions (*i.e.*, a 941 base pair segment of the mitochondrial cytochrome oxidase I and II genes, a 171 base pair segment of β -tubulin, and a 921 base pair segment of elongation factor-1 α) all showed deep between clade divergence and low within clade variation, which suggests that the clades represent distinct species.

Isolating Mechanism

In laboratory studies, Reitz and Trumble (2002) observed a higher frequency of homotypic than heterotypic mating in two Californian *L. huidobrensis* populations that do not interbreed in nature (Morgan *et al.*, 2000). Reitz and Trumble (2002) did not find any relationship between the type of mating (*i.e.*, homotypic or heterotypic) and the number of offspring produced, so they concluded that the natural reproductive isolating mechanism must occur prior to mating. Observations of mating behavior in their laboratory colonies indicate that females choose a mate and “aggressively kick” at undesirable males (Reitz and Trumble, 2002). Their data also indicate that females are more likely to mate on certain hosts; homotypic and heterotypic pairs successfully mated more frequently on celery than on pepper (Reitz and Trumble, 2002). Given differences in the known host ranges of Californian and Latin American *L. huidobrensis* populations (see Ecology above) and the many reasons that a female may choose one potential mate over others (see Alcock, 2001), it seems likely that the Californian and Latin American populations may not interbreed if they meet in nature.

References

- Alcock, J. 2001. The Evolution of Reproductive Behavior. Pages 317-358 in J. Alcock, (ed.). Animal Behavior. Sinauer Associates, Inc, Sunderland, Massachusetts.
- Ameixa, O., L. Almeida, A. Gonçalves, and L. Neto. 2007. Feeding behavior of *Liriomyza huidobrensis* (Blanchard) and *L. trifolii* (Burgess) adults on bean leaves. Journal of Insect Behavior. 20(1).
- Blanchard, E. 1926. A dipterous leaf-miner on *Cineraria*, new to science. Revista de la Sociedad Entomológica Argentina. 1: 10-11.
- Borchert, D. M. 2006. Organism Pest Risk Assessment of Pea Leafminer, *Liriomyza huidobrensis*. USDA, PPQ, CPHST, PERAL, Raleigh, North Carolina.
- CABI. 2006. Crop Protection Compendium. CAB International, Wallingford, United Kingdom [CD-ROM].
- CDFA. 2006. California Agricultural Resource Directory 2006. California Department of Food and Agriculture, Sacramento, California. 176 pp.

Attachment 2-SPRO-CPHST report

- Chen, B., and L. Kang. 2004. Variation in cold hardiness of *Liriomyza huidobrensis* (Diptera: Agromyzidae) along latitudinal gradients. *Environmental Entomology*. 33(2): 155-164.
- Civelek, H. S., Z. Yoldaş, and M. R. Ulusoy. 2004. Seasonal population trends of *Liriomyza huidobrensis* (Blanchard, 1926) (Diptera: Agromyzidae) on cucumber (*Cucumis sativus* L.) in Western Turkey. *Journal of Pest Science*. 77: 85-89.
- Colpetzer, K. 2007. *Liriomyza huidobrensis*. Personal communication to William E. Chaney (UC Davis), Phil A. Phillips (UC Davis), Karen L. Robb (UC Davis), and John Trumble (UC Riverside) on 07 June 2007 from Keith Colpetzer (PPQ). Archived at the PERAL library, Raleigh, NC.
- EPPO. 2005. *Liriomyza* spp. EPPO Bulletin. 35(2): 335-344.
- Gary, W. J., D. F. Mayer, and A. L. Antonelli. 1986. Pea leafminer: Extension Bulletin 1372E. Washington State University, Pullman, WA.
- Górski, R. 2005. Effectiveness of natural essential oils in monitoring of the occurrence of pea leafminer (*Liriomyza huidobrensis* Blanchard) in *Gerbera* crop. *Journal of Plant Protection Research*. 45(4): 287-291.
- Halbert, S. E. 2006. Federal and state plant protection and quarantine. *Tri-ology*. 45(5): 11-12.
- Hammad, E. M. A.-F., N. M. Nemer, and N. S. Kawar. 2000. Efficacy of Chinaberry tree (Meliaceae) aqueous extracts and certain insecticides against the pea leafminer (Diptera: Agromyzidae). *Journal of Agricultural Science*. 134: 413-420.
- Hardy, D. E., and M. D. Delfinado. 1980. Family Agromyzidae. Pages 191-222 in E. C. Zimmerman, (ed.). *Insects of Hawaii*, Volume 13. The University Press of Hawaii, Honolulu, Hawaii.
- He, L., Y. Zhang, N. Xiao, JianingWei, and R. Kuang. 2002. *Liriomyza huidobrensis* in Yunnan, China: current distribution and genetic structure of a recently established population. *Entomologia Experimentalis et Applicata*. 102: 213-219.
- Hidayani, Purnomo, A. Rauf, P. M. Ridland, and A. A. Hoffmann. 2005. Pesticide applications on Java potato fields are ineffective in controlling leafminers, and have antagonistic effects on natural enemies of leafminers. *International Journal of Pest Management*. 51(3): 181-187.
- Kox, L. F. F., H. E. van-den-Beld, B. I. Lindhout, and L. J. W. de-Goffau. 2005. Identification of economically important *Liriomyza* species by PCR-RFLP analysis. EPPO Bulletin. 35(1): 79-85.
- Lange, W. H., A. A. Grigarick, and E. C. Carlson. 1957. Serpentine leafminer damage. *California Agriculture*. 11: 3-5.
- Lanzoni, A., G. G. Bazzocchi, G. Burgio, and M. R. Fiacconi. 2002. Comparative life history of *Liriomyza trifolii* and *Liriomyza huidobrensis* (Diptera: Agromyzidae) on beans: Effect of temperature on development. *Environmental Entomology*. 31(5): 797-803.
- Lodge, D. M. 1993. Biological Invasions: Lessons for ecology. *Trends in Ecology and Evolution*. 8(4): 133-137.
- Marshall, C. J. 2007. RE: *Liriomyza huidobrensis*. Personal communication to Keith Colpetzer (PPQ) on 28 May 2007 from Christopher Marshall (OSU). Archived at the PERAL library, Raleigh, NC.
- Martin, A. D., R. H. Hallett, M. K. Sears, and M. R. McDonald. 2005a. Overwintering ability of *Liriomyza huidobrensis* (Blanchard) (Diptera: Agromyzidae) in southern Ontario, Canada. *Environmental Entomology*. 34(4): 743-747.
- Martin, A. D., D. Stanley-Horn, and R. H. Hallett. 2005b. Adult host preference and larval performance of *Liriomyza huidobrensis* (Diptera: Agromyzidae) on selected hosts. *Environmental Entomology*. 34(5): 1170-1177.
- Masetti, A., A. Luchetti, B. Mantovani, and G. Burgio. 2006. Polymerase chain reaction-restriction fragment length polymorphism assays to distinguish *Liriomyza huidobrensis* (Diptera: Agromyzidae) from associated species on lettuce cropping systems in Italy. *Journal of Economic Entomology*. 99(4): 1268-1272.
- Mayr, E. 1996. What is a species, and what is not? *Philosophy of Science*. 63(2): 262-277.
- Morgan, D. J. W., S. R. Reitz, P. W. Atkinson, and J. T. Trumble. 2000. The resolution of Californian populations of *Liriomyza huidobrensis* and *Liriomyza trifolii* (Diptera: Agromyzidae) using PCR. *Heredity*. 85: 53-61.
- NASS. 2007. USDA National Agricultural Statistics Service. Last accessed 15 May 2007, <http://www.nass.usda.gov/index.asp>.
- NOAA. 2007. NOAA Online Weather Data. National Oceanic and Atmospheric Administration. Last accessed 10 May 2007, <http://www.dnr.sc.gov/climate/sercc/nowdata.html>.
- Parrella, M. P. 1987. Biology of *Liriomyza*. *Annual Review of Entomology*. 32: 201-224.
- Parrella, M. P., and J. A. Bethke. 1984. Biological studies of *Liriomyza huidobrensis* (Diptera: Agromyzidae) on chrysanthemum, aster, and pea. *Journal of Economic Entomology*. 77(2): 342-345.

Attachment 2-SPRO-CPHST report

- Parrella, M. P., V. P. Jones, R. R. Youngman, and L. M. Lebeck. 1985. Effect of leaf mining and leaf stippling of *Liriomyza* spp. on photosynthetic rates of chrysanthemum. *Annals of the Entomological Society of America*. 78(1): 90-93.
- Pelter, G. Q. 2003. Onion leaf injury by pea leafminer (*Liriomyza huidobrensis*). USDA Cooperative Extension Washington State University. Last accessed 22 May 2007, http://grant-adams.wsu.edu/agriculture/agrifocus_newsletters/2003/September2003Agri-Focus.pdf.
- Poe, S. L., and J. K. Montz. 1981. Preliminary results of a leafminer species survey. Pages 24-34 in D. J. Schuster, (ed.). Proceedings of the University of Florida, Institute of Food and Agricultural Sciences-Industry Conference on Biology and Control of *Liriomyza* Leafminers, Lake Buena Vista, Florida.
- Rauf, A., B. M. Shepard, and M. W. Johnson. 2000. Leafminers in vegetables, ornamental plants and weeds in Indonesia: surveys of host crops, species composition and parasitoids. *International Journal of Pest Management*. 46(4).
- Reitz, S. R., and J. T. Trumble. 2002. Interspecific and intraspecific differences in two *Liriomyza* leafminer species in California. *Entomologia Experimentalis et Applicata*. 102: 101-113.
- Scheffer, S. J. 2000. Molecular evidence of cryptic species within the *Liriomyza huidobrensis* (Diptera: Agromyzidae). *Journal of Economic Entomology*. 93(4): 1146-1151.
- Scheffer, S. J., and M. L. Lewis. 2001. Two nuclear genes confirm mitochondrial evidence of cryptic species within *Liriomyza huidobrensis* (Diptera: Agromyzidae). *Annals of the Entomological Society of America*. 94(5): 648-653.
- Scheffer, S. J., M. L. Lewis, and R. C. Joshi. 2006. DNA barcoding applied to invasive leafminers (Diptera: Agromyzidae) in the Philippines. *Annals of the Entomological Society of America*. 99(2): 204-210.
- Scheffer, S. J., A. Wijesekara, D. Visser, and R. H. Hallett. 2001. Polymerase chain reaction-restriction fragment-length polymorphism method to distinguish *Liriomyza huidobrensis* from *L. langei* (Diptera: Agromyzidae) applied to three recent leafminer invasions. *Journal of Economic Entomology*. 94(5): 1177-1182.
- Shepard, B. M., Samsudin, and A. R. Braun. 1998. Seasonal incidence of *Liriomyza huidobrensis* (Diptera: Agromyzidae) and its parasitoids on vegetables in Indonesia. *International Journal of Pest Management*. 44(1): 43-47.
- Shiao, S.-F. 2004. Morphological diagnosis of six *Liriomyza* species (Diptera: Agromyzidae) of quarantine importance in Taiwan. *Applied Entomology and Zoology*. 39(1): 27-39.
- Shiao, S.-F., and W.-J. Wu. 2000. *Liriomyza huidobrensis* (Blanchard), a newly invaded insect of economic importance to Taiwan (Diptera: Agromyzidae). *Plant Protection Bulletin*. 42(4): 249-254.
- Spencer, K. A. 1973. Agromyzidae (Diptera) of Economic Importance. Dr. W. Junk B.V., The Hague, Denmark.
- Spencer, K. A. 1990. Host Specialization in the World Agromyzidae (Diptera). Kluwer Academic Publishers, Dordrecht, The Netherlands.
- Steck, G. J., and W. N. Dixon. 2006. California pea leafminer, *Liriomyza langei* (Diptera: Agromyzidae). Florida Department of Agriculture and Consumer Services. Last accessed 25 April 2007, <http://www.doacs.state.fl.us/pi/enpp/ento/pealeafminer.html>.
- Stegmaier Jr., C. E. 1968. A review of recent literature on the host plant range of the genus *Liriomyza* Mik (Diptera: Agromyzidae) in the continental United States and Hawaii, excluding Alaska. *Florida Entomologist*. 51(3): 167-182.
- USDA-NRCS. 2007. The PLANTS Database. Last accessed 22 May 2007, <http://plants.usda.gov/>.
- Wei, J., L. Zou, R. Kuang, and L. He. 2000. Influence of leaf tissue structure on host feeding selection by pea leafminer *Liriomyza huidobrensis* (Diptera: Agromyzidae). *Zoological Studies*. 39(4): 295-300.
- Weintraub, P. G. 2001. Changes in the dynamics of the leafminer, *Liriomyza huidobrensis*, in Israeli potato fields. *International Journal of Pest Management*. 47(2): 95-102.
- Weintraub, P. G., and A. R. Horowitz. 1995. The newest leafminer pest in Israel, *Liriomyza huidobrensis*. *Phytoparasitica*. 23(4): 177-184.
- Weintraub, P. G., and A. R. Horowitz. 1996. Spatial and diel activity of the pea leafminer (Diptera: Agromyzidae) in potatoes, *Solanum tuberosum*. *Environmental Entomology*. 25(4): 722-726.